

Appln # 09/548,998  
Paper # 27 Attach.

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(FILE 'HOME' ENTERED AT 20:23:33 ON 18 MAR 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE' ENTERED AT 20:23:39 ON 18 MAR 2003

L1 29182 16S RDNA OR 16S RRNA  
L2 4048 (DECHLORINATING OR DECHLORINATION) (P) 1  
L3 104 (DECHLORINATING OR DECHLORINATION) (N) BACTERIA  
L4 2 L1 (P) L3  
L5 2 DUP REM L4 (0 DUPLICATES REMOVED)  
L6 88 (EBERSOLE R? OR EBERSOLE, R?)/AU,IN  
L7 337 (HENDRICKSON E? OR HENDRICKSON, E?)/AU,IN  
L8 11 L1 AND (L6 OR L7)  
L9 5 DUP REM L8 (6 DUPLICATES REMOVED)  
L10 20034 RIBOSOMAL DNA  
L11 45463 RIBOSOMAL RNA  
L12 66 DEHALOBACTER  
L13 44 (DECHLORINATING OR DECHLORINATION) (S) L12  
L14 0 L10 (P) L13  
L15 4 L10 AND L13  
L16 0 L11 (P) L13  
L17 4 DUP REM L15 (0 DUPLICATES REMOVED)

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L5 ANSWER 1 OF 2 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

AB. . . several years. Some pure cultures are capable of gaining energy from the dechlorination process. However, little is known about the dechlorinating bacteria and their kinetics at groundwater temperature, which is typically 10degreeC in the Pacific Northwest. Therefore, we established a PCE to. . . as alternative electron acceptors that supported growth of JPD-1. JPD-1 did not have catalase but tested positive for oxidase. The 16S rDNA sequence of JPD-1 was 98% similar to Sulfurospirillum sp. DSM 806 and Dehalospirillum multivorans, a known PCE to cDCE dechlorinator.. . . appears to be a different species. Furthermore, the ability of JPD-1 to grow under microaerophilic conditions suggests that other microaerophilic dechlorinating bacteria may exist. This might explain why in so many environmental sites PCE is found to readily transform to cDCE.

ACCESSION NUMBER: 2002:608755 BIOSIS

DOCUMENT NUMBER: PREV200200608755

TITLE: Characterization of a microaerophilic and psychrotrophic tetrachloroethene to cis-1,2-dichloroethene dechlorinating bacterium, strain JPD-1.

AUTHOR(S): Pietari, J. M. H. (1); Herwig, R. P. (1); Ferguson, J. F. (1)

CORPORATE SOURCE: (1) University of Washington, Seattle, WA USA

SOURCE: Abstracts of the General Meeting of the American Society for Microbiology, (2002) Vol. 102, pp. 401.  
<http://www.asmtg.org/mtgsrc/generalmeeting.htm>. print.

Meeting Info.: 102nd General Meeting of the American  
Society for Microbiology Salt Lake City, UT, USA May 19-23,  
2002 American Society for Microbiology  
. ISSN: 1060-2011.

DOCUMENT TYPE: Conference  
LANGUAGE: English

L5 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS

AB . . . bioremediation of 1,2-dichloropropane (DCP)-contaminated sites  
and wastewater streams was analyzed. A direct culture-dependent approach  
based on in vitro amplification of 16S rRNA genes was  
chosen for complex microbiota detn. The occurrence of 16S  
rDNA sequences closely related to cultivated  
dechlorinating bacteria and to yet-uncultured bacteria  
of other anaerobic, dechlorinating microbial communities give evidence for  
a specific community structure.

ACCESSION NUMBER: 2000:283645 CAPLUS

DOCUMENT NUMBER: 132:325434

TITLE: Molecular characterization of 1,2-dichloropropane  
transforming mixed cultures

AUTHOR(S): Schlotelburg, Cord; Von Wintzingerode, Friedrich;  
Hauck, Regine; Hegemann, Werner; Gobel, Ulf B.

CORPORATE SOURCE: Humboldt-Universitat zu Berlin Institut fur  
Mikrobiologie und Hygiene, Berlin, 10117, Germany

SOURCE: Biologische Abwasserreinigung (1999), 12(Behandlung  
von Abwaessern mit Halogen-Organischen Verbindungen),  
135-146

CODEN: BABWEK; ISSN: 0941-8806

PUBLISHER: Technische Universitaet Berlin

DOCUMENT TYPE: Journal

LANGUAGE: English

REFERENCE COUNT: 21 THERE ARE 21 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 1 OF 5 MEDLINE DUPLICATE 1

AB A laboratory microcosm study and a pilot scale field test were conducted  
to evaluate biostimulation and bioaugmentation to dechlorinate  
tetrachloroethene (PCE) to ethene at Kelly Air Force Base. The site  
groundwater contained about 1 mg/L of PCE and lower amounts of  
trichloroethene (TCE) and cis-1,2-dichloroethene (cDCE). Laboratory  
microcosms inoculated with soil and groundwater from the site exhibited  
partial dechlorination of TCE to cDCE when amended with lactate or  
methanol. Following the addition of a dechlorinating enrichment culture,  
KB-1, the chlorinated ethenes in the microcosms were completely converted  
to ethene. The KB-1 culture is a natural dechlorinating microbial  
consortium that contains phylogenetic relatives of Dehalococcoides  
ethenogenes. The ability of KB-1 to stimulate biodegradation of  
chlorinated ethenes in situ was explored using a closed loop recirculation  
cell with a pore volume of approximately 64,000 L. The pilot test area  
(PTA) groundwater was first amended with methanol and acetate to establish  
reducing conditions. Under these conditions, dechlorination of PCE to cDCE  
was observed. Thirteen liters of the KB-1 culture were then injected into  
the subsurface. Within 200 days, the concentrations of PCE, TCE, and  
cis-1,2-DCE within the PTA were all below 5 microg/L, and ethene

production accounted for the observed mass loss. The maximum rates of dechlorination estimated from field data were rapid (half-lives of a few hours). Throughout the pilot test period, groundwater samples were assayed for the presence of Dehalococcoides using both a Dehalococcoides-specific PCR assay and 16S rDNA sequence information. The sequences detected in the PTA after bioaugmentation were specific to the Dehalococcoides species in the KB-1 culture. These sequences were observed to progressively increase in abundance and spread downgradient within the PTA. These results confirm that organisms in the KB-1 culture populated the PTA aquifer and contributed to the stimulation of dechlorination beyond cDCE to ethene.

ACCESSION NUMBER: 2003016643 IN-PROCESS

DOCUMENT NUMBER: 22410886 PubMed ID: 12523427

TITLE: Field demonstration of successful bioaugmentation to achieve dechlorination of tetrachloroethene to ethene.

AUTHOR: Major David W; McMaster Michaye L; Cox Evan E; Edwards Elizabeth A; Dworatzek Sandra M; Hendrickson Edwin R; Starr Mark G; Payne Jo Ann; Buonamici Lois W

CORPORATE SOURCE: GeoSyntec Consultants, Inc., 130 Research Lane, Suite 2 Guelph, Ontario, N1G 5G3 Canada.. dmajor@geosyntec.com

SOURCE: ENVIRONMENTAL SCIENCE & TECHNOLOGY, (2002 Dec 1) 36 (23) 5106-16.

Journal code: 0213155. ISSN: 0013-936X.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: IN-PROCESS; NONINDEXED; Priority Journals

ENTRY DATE: Entered STN: 20030114

Last Updated on STN: 20030114

L9 ANSWER 2 OF 5 MEDLINE

DUPLICATE 2

AB The environmental distribution of Dehalococcoides group organisms and their association with chloroethene-contaminated sites were examined. Samples from 24 chloroethene-dechlorinating sites scattered throughout North America and Europe were tested for the presence of members of the Dehalococcoides group by using a PCR assay developed to detect Dehalococcoides 16S rRNA gene (rDNA) sequences. Sequences identified by sequence analysis as sequences of members of the Dehalococcoides group were detected at 21 sites. Full dechlorination of chloroethenes to ethene occurred at these sites. Dehalococcoides sequences were not detected in samples from three sites at which partial dechlorination of chloroethenes occurred, where dechlorination appeared to stop at 1,2-cis-dichloroethene. Phylogenetic analysis of the 16S rDNA amplicons confirmed that Dehalococcoides sequences formed a unique 16S rDNA group. These 16S rDNA sequences were divided into three subgroups based on specific base substitution patterns in variable regions 2 and 6 of the Dehalococcoides 16S rDNA sequence. Analyses also demonstrated that specific base substitution patterns were signature patterns. The specific base substitutions distinguished the three sequence subgroups phylogenetically. These results demonstrated that members of the Dehalococcoides group are widely distributed in nature and can be found in a variety of geological formations and in different climatic zones. Furthermore, the association of these organisms with full dechlorination of chloroethenes suggests that they are promising candidates for engineered bioremediation and may be important contributors to natural

attenuation of chloroethenes.

ACCESSION NUMBER: 2002108917 MEDLINE  
 DOCUMENT NUMBER: 21681025 PubMed ID: 11823182  
 TITLE: Molecular analysis of Dehalococcoides 16S ribosomal DNA  
 from chloroethene-contaminated sites throughout North  
 America and Europe.

AUTHOR: Hendrickson Edwin R; Payne Jo Ann; Young Roslyn  
 M; Starr Mark G; Perry Michael P; Fahnestock Stephen; Ellis  
 David E; Ebersole Richard C

CORPORATE SOURCE: Corporate Center for Engineering Research, Central Research  
 and Development, E. I. Du Pont de Nemours & Co., Glasgow  
 Site 300/318, Newark, DE 19714, USA..  
 edwin.r.hendrickson@usa.dupont.com

SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (2002 Feb) 68 (2)  
 485-95.  
 Journal code: 7605801. ISSN: 0099-2240.

PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals

OTHER SOURCE: GENBANK-AF388530; GENBANK-AF388531; GENBANK-AF388532;  
 GENBANK-AF388533; GENBANK-AF388534; GENBANK-AF388535;  
 GENBANK-AF388536; GENBANK-AF388537; GENBANK-AF388538;  
 GENBANK-AF388539; GENBANK-AF388540; GENBANK-AF388541;  
 GENBANK-AF388542; GENBANK-AF388543; GENBANK-AF388544;  
 GENBANK-AF388545; GENBANK-AF388546; GENBANK-AF388547;  
 GENBANK-AF388548; GENBANK-AF388549; GENBANK-AF388550

ENTRY MONTH: 200203  
 ENTRY DATE: Entered STN: 20020214  
 Last Updated on STN: 20020403  
 Entered Medline: 20020328

L9 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2003 ACS

AB The Bioremediation Consortium of the Remediation Technologies Development  
 Forum (RTDF) carried out a successful anaerobic bioaugmentation pilot to  
 bioremediate a chloroethene tetrachloroethene (PCE), trichloroethene (TCE)  
 and cis-1,2-dichloroethene (cDCE) contaminated aquifer at Kelly Air Force  
 Base near San Antonio, Texas. An anaerobic dechlorinating enrichment  
 culture, KB-1, was injected into the ground to duplicate the successful  
 bioaugmentation pilot at Dover AFB, Delaware (DE) (1997-1999). The KB-1  
 community structure has been analyzed and shown to have a Dehalococcoides  
 ethenogenes-like species present in its community structure. D.  
 ethenogenes (DHE) is an organism described by Maymo-Gatell et al. (1997).  
 DHE was shown to dechlorinate PCE and TCE by removing all the Cl atoms to  
 form ethene, through a process known as dehalorespiration. D.  
 ethenogenes-like (DHE-like) organisms detected in samples from .apprx.30  
 different sites in North America and Europe have shown 16S  
 rRNA gene sequences (rDNA) with signature sequences that are  
 unique to the sampling site. This was found true for the KB-1 DHE-like  
 organism. We have developed a specific 16S rRNA  
 polymerase chain reaction (PCR) assay to detect DHE-like organisms. Using  
 the PCR assay and 16S rDNA sequence information,  
 groundwater samples were monitored during the course of the Kelly pilot  
 study. The DHE-like organism was not detected in the control groundwater  
 that had been given electron donors (methanol and acetate). After  
 bioaugmentation, PCR anal. of groundwater from monitoring wells detected

the DHE-like organism. Detection 1st appeared in the injection well and then in down gradient monitoring wells (1st in the nearest well and then in the well wells further down gradient). The DHE-like organism was detected in the extn. wells, 2 mo after bioaugmentation. Together, with field data from monitoring wells that have demonstrated dechlorination of PCE to ethene, the PCR and sequence data suggest that the bioaugmentation culture, KB-1, had colonized the test plot in the Kelly AFB chloroethene contaminated aquifer.

ACCESSION NUMBER: 2002:127852 CAPLUS

DOCUMENT NUMBER: 136:406288

TITLE: Using a molecular approach to monitor a  
bioaugmentation pilot

AUTHOR(S): Hendrickson, Edwin R.; Starr, Mark G.;  
Elbersen, Margaret A.; Payne, Jo Ann.; Mack, E. Erin;  
Huang, Hui-Bin; McMaster, Michaye L.; Ellis, David E.

CORPORATE SOURCE: DuPont Co., Newark, DE, USA

SOURCE: International In Situ and On-Site Bioremediation  
Symposium, 6th, San Diego, CA, United States, June  
4-7, 2001 (2001), Volume 8, 43-51. Editor(s): Leeson,  
Andrea. Battelle Press: Columbus, Ohio.  
CODEN: 69CHBZ; ISBN: 1-57477-110-8

DOCUMENT TYPE: Conference

LANGUAGE: English

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L9 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2003 ACS

AB A unique 16S rRNA profile derived from Dehalococcoides  
ethenogenes and that is assocd. with dechlorination activity has been  
identified and isolated. The profile contains a nucleic acid fragment  
that is linked to dechlorinating activity. Isolation of D. ethenogenes  
strains and correlation of 16S rRNA polymorphisms with  
dechlorination activity is demonstrated.

ACCESSION NUMBER: 2000:756923 CAPLUS

DOCUMENT NUMBER: 133:319474

TITLE: A 16S rRNA sequence for  
identification of the dechlorinating strains of the  
bacterium Dehalococcoides ethenogenes

INVENTOR(S): Hendrickson, Edwin R.; Ebersole, Richard C.

PATENT ASSIGNEE(S): E.I. Du Pont De Nemours and Co., USA

SOURCE: PCT Int. Appl., 55 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
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WO 2000063443	A2	20001026	WO 2000-US9883	20000413
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WO 2000063443	A3	20020510		
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W: CA, MX, US

RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,  
PT, SE

EP 1224323	A2	20020724	EP 2000-922139	20000413
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R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,

IE, FI, CY  
PRIORITY APPLN. INFO.: US 1999-129511P P 19990415  
WO 2000-US9883 W 20000413

L9 ANSWER 5 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 2000:403608 BIOSIS

DOCUMENT NUMBER: PREV200000403608

TITLE: Detection and characterization of Dehalococcoides  
ethenogenes' 16S rRNA sequences found  
in groundwater and soils from waste sites contaminated with  
PCE and TCE.

AUTHOR(S): Elberson, M. A. (1); Tabinowski, J. A. (1); Ebersole,  
R. C. (1); Ellis, D. E.; Hendrickson, E. R.  
(1)

CORPORATE SOURCE: (1) Leveraged Biology Group, BCSE, CRD, DuPont Co., Newark,  
DE USA

SOURCE: Abstracts of the General Meeting of the American Society  
for Microbiology, (2000) Vol. 100, pp. 568. print.  
Meeting Info.: 100th General Meeting of the American  
Society for Microbiology Los Angeles, California, USA May  
21-25, 2000 American Society for Microbiology  
. ISSN: 1060-2011.

DOCUMENT TYPE: Conference

LANGUAGE: English

SUMMARY LANGUAGE: English

## Loeb, Bronwen

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**From:** Loeb, Bronwen  
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Appln 09/548,998

Schlotelburg et al (1999) "Molecular characterization of 1,2-dichloropropane transforming mixed cultures" *Biologische Abwasserreinigung* (1999), 12(Behandlung von Abwaessern mit Halogen-Organischen Verbindungen), pages 135-146

von Wintzingerode et al (1999) *Applied and Environmental Microbiology*, (Jan., 1999) Vol. 65, No. 1, pp. 283-286.

L Number	Hits	Search Text	DB	Time stamp
1	1406	"16s rRNA" or "16s rDNA"	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/03/18 21:12
3	2	dehalobacter	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/03/18 21:12
4	1	((dechlorination or dechlorinating) near bacteri\$) same ("16s rRNA" or "16s rDNA")	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/03/18 21:12
5	3	("16s rRNA" or "16s rDNA") and ((dechlorination or dechlorinating) near bacteri\$)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/03/18 21:12
2	18	(dechlorination or dechlorinating) near bacteri\$	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/03/18 21:14
6	0	(identify or "signature sequence" or identifier) near ((dechlorination or dechlorinating) near bacteri\$)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/03/18 21:15
7	0	(identify or "signature sequence" or identifier) with ((dechlorination or dechlorinating) near bacteri\$)	USPAT; US-PGPUB; EPO; JPO; DERWENT	2003/03/18 21:15